REMARKS

Status of the claims

Claims 1, 6-12, 15, 19-25, and 28 are pending. Claims 1, 6-12, 15, 19-25, and 28 are rejected. Claims 1, 15 and 28 are amended herein. Claims 2-5, 11, 13-14, 16-18, 23, and 26-27 are/were canceled. No new matter has been added.

Amendments to the claims

Independent claims 1 and 15 are amended to overcome rejections under 35 U.S.C. 102(a)(b)(e) over **Pitner** *et al.* and **Jayasena** *et al.* The limitations of claims 11 and 23, are incorporated into claims 1 and 15, respectively, to recite that the ligand is a molecule that is not a nucleic acid sequence bound by the signaling aptamer (pg. 15, ll. 7-9), as further discussed *infra*. Claims 1 and 15 are amended further to recite that the reporter/dye is covalently coupled in proximity to a binding site specific for the ligand (pg. 21, ll. 9+; Figs. 1A-1B, 2). Claim 28 is amended to more clearly indicate the method step is a further method step of amended independent claim 15. Claims 11 and 23 are canceled. No new matter has been added.

The 35 U.S.C. §102(b) rejection

Claims 1, 6-10, 12, 15, 19, 25, and 28 are rejected under 35 U.S.C. §102(b) as being anticipated by **Pitner** *et al.* (U.S. Patent No. 5,691,145). Applicant respectfully traverses this rejection.

The Examiner states that, regarding claims 1 and 15, **Pitner** *et al.* disclose a method of transducing a conformation change of a signaling aptamer, e.g., G-quartet (col. 2, ll. 31-50) that occurs upon the signaling aptamer binding a ligand to a detectable increased signal generated by a reporter molecule (labeled nucleotide) that is coupled to the aptamer prior to binding (col. 5, ll. 15-47). The Examiner states the method comprises covalently coupling the reporter molecule/fluorescent dye to an aptamer to form a signaling aptamer where the reporter/fluorescent dye replaces a nucleic acid within the aptamer (col. 5, ll. 16-17; 21-24; and 26-28), placing the signaling aptamer in solution, contacting the signaling aptamer in solution with the ligand under conditions whereby the aptamer binds the ligand and detecting the increase in fluorescence intensity generated by the reporter/fluorescent dye transduced by conformational change in the signaling aptamer upon binding the ligand (col. 7, ll. 31-59).

Pitner *et al.* teach labeling an oligonucleotide G-quartet structure with a donor fluorophore and an acceptor dye at positions where the G-quartet structure brings the donor and acceptor in proximity so that the acceptor dye quenches the donor fluorophor (Abstract). The labeled structure is useful for detecting or identifying nucleotide sequences (col. 2, ll. 65-68). In the presence of a complementary nucleotide the G-quartet unfolds or linearizes to hybridize therewith (col. 3, ll. 9-10). Unfolding or linearizing separates the donor fluorophore and acceptor dye sufficiently to decrease donor quenching and increase donor fluorescence (col. 3, ll. 11-15).

Applicant's invention as recited in amended claims 1 and 15 is discussed *supra*. Specifically, the limitations of claims 11 and 23 were incorporated into these claims to limit the ligands to those that do not have nucleic acid sequences. The ligands in **Pitner**

et al. specifically are nucleic acid sequences. Thus, **Pitner** *et al.* do not teach this claim element.

Claims 6-10 and 12 depend from amended claim 1 and claims 19-22, 24-25 and 28 depend from amended claim 15. These claims further limit the type of reporter molecule or aptamer, the state of the ligand and, for amended claim 15, further limit the method by correlating fluorescence increase with amount of ligand. The incorporation of the limits of any of these claims into their respective independent claims would not be anticipated by **Pitner** *et al.* because the claim elements not anticipated by **Pitner** *et al.* in these independent claims are not limited by claims 6-10, 12, 19-22, 24-25 and 28.

Absent teachings of non-nucleic acid ligands, **Pitner** *et al.* does not anticipate the amended claims. Accordingly, in view of the amendments and arguments presented herein, Applicants respectfully request that the rejection of claims 1, 6-10, 12, 15, 19, 25, and 28 under 35 U.S.C. §102(b) be withdrawn.

The 35 U.S.C. §102(a) & (e) rejections

Claims 1, 6-12, 15, 19, 23, 25, and 28 are rejected under 35 U.S.C. §102(a) as being anticipated by **Jayasena** *et al.* (WO 99/31276). Claims 1, 6-12, 15, 19, 23, 25, and 28 are rejected under 35 U.S.C. §102(e) as being anticipated by **Jayasena** *et al.* (U.S. 6,531,286). Applicant respectfully traverses these rejections. In presenting arguments *infra*, Applicant is referring to both **Jayasena** *et al.* ('31276) and **Jayasena** *et al.* ('286). Any reference to a specific teaching can be found exactly in both and Applicant will include place citations of a particular teaching for both '31276 (pg./lines) and '286 (col./lines).

For independent claims 1 and 15, the Examiner maintains that **Jayasena** *et al.* teach Applicant's method of transducing a conformational change of a signaling aptamer that occurs upon binding a ligand to a detectable increased signal generated by a reporter molecule/fluorescent dye appended to the aptamer prior to binding. The Examiner also states that fluorescein phosphoramidite is covalently coupled to form the signaling aptamer by replacing a nucleic acid in the aptamer (pg. 43, ll. 12-27; col. 29, ll. 40-65) to form a signaling aptamer wherein the reporter/dye a nucleic acid in the aptamer.

The Examiner also maintains that **Jayasena** *et al.* teach placing the signaling aptamer in solution, contacting the signaling aptamer in solution with the ligand under conditions whereby the aptamer binds the ligand and detecting the increase in fluorescence intensity generated by the reporter/dye transduced by conformational change in the signaling aptamer upon binding the ligand (pg. 32, ll. 14 to pg. 34, ll. 30; col. 23, ll. 7-64; Claim 1). Furthermore, the Examiner states that **Jayasena** *et al.* teach the reporter molecule/dye is coupled to the aptamer within the aptamer (pg. 28, ll. 20-24; col. 19, l. 40).

Jayasena *et al.* teach a ligand beacon assay where a molecular beacon is the reporter and nucleic acid ligands are the sensor (Abstract). The molecular beacon sequence contains at least one fluorescent group, e.g., fluorescein, and at least one quenching moiety, e.g., DABCYL (pg. 26, ll. 24-26; col. 18, ll. 23-27; Fig. 1). The sequence of the molecular beacon contains a loop that is complementary to a nucleic acid ligand that is an aptamer (pg. 29, ll. 17-20; col. 8, ll. 25-29; Fig. 2A). The molecular beacon can hybridize to nucleic acid ligands or aptamers that are either free of their cognate targets and do not bind their targets once hybridized (pg. 29, ll. 17+ or col. 8, ll. 25-47; pg. 29, ll. 20-21 or col. 20, ll. 18-20) or to a nucleic acid ligand that has undergone a conformational change in the presence of

the target to bind the target subsequently allowing hybridization of the ligand to the ligand beacon (pg. 29, ll. 23-31; col. 20, ll. 21-42). In either case configuration of the nucleic acid ligand must change to hybridize to the ligand beacon which is accompanied by a measurable change in the spectral properties of the molecular beacon (pg. 8, ll. 7-8; col. 6, ll. 10-13).

The Examiner, in responding to Applicant's arguments filed in the response to the previous Office Action mailed 10/27/03, states that the molecular beacon in **Jayasena** *et al.* is a signaling aptamer because a reporter/dye is covalently coupled within a nucleic acid comprising a stem loop. Thus, according to the Examiner, **Jayasena** *et al.* teach contacting the signaling aptamer (molecular beacon) with a ligand (target) and detecting fluorescence resulting from ligand-aptamer binding via hybridization (pg. 8, ll. 14-21; col. 10, ll. 10-24). In rebutting Applicant's argument, the Examiner stated that the instant claim language "comprising" encompasses the binding via hybridization. The claims do not require "direct" binding of the ligand-aptamer.

Other than teaching each and every element of the claimed invention, anticipation of a claim requires that the elements in the prior art must be arranged as required by the claim. First, in stating that the molecular beacon is a signaling aptamer because a reporter/dye is covalently coupled within a nucleic acid sequence, the Examiner is not considering the quenching moiety that must comprise the beacon for it to function. Applicant's amended claims do not recite that the signaling aptamer comprises an aptamer and a reporter/dye covalently coupled therein. With regard to the signaling aptamer the claim language is not open-ended and the signaling aptamer could not encompass other moieties. In Applicant's invention positioning of the reporter/dye alone within the

aptamer "quenches" the fluorescence. **Jayasena** *et al.* specifically teach a fluorescent moiety and a quenching moiety (pg. 21, ll. 6-7; col. 14, ll. 37-43).

Second, amended claims 1 and 15 require that a signaling aptamer have a reporter/dye in proximity to a binding site specific for the ligand (target) and that the ligand is a molecule bound by the signaling aptamer where the ligand is not a nucleic acid sequence (pg. 15, ll. 7-11). The amended claims further recite binding the ligand to the binding site of the signaling aptamer. Therefore, the binding site in Applicant's signaling aptamer cannot bind a nucleic acid sequence which precludes binding via hybridization. Thus, if the ligand beacon in **Jayasena** *et al.* is a signaling aptamer, it cannot hybridize to a nucleic acid ligand bound to the target and anticipate the instant invention.

As taught by **Jayasena** *et al.*, the molecular beacon or ligand beacon is the reporter molecule and the nucleic acid ligand is the aptamer where the aptamer binds the target (pg. 16, ll. 16-17 or col. 11, ll. 26-27; pg. 17, ll. 15 or col. 12, ll. 3-4). **Jayasena** *et al.* teach that the ligand beacon is any nucleic acid that can hybridize to a nucleic acid ligand and in doing so undergo a conformational change that alters the distance between nucleotides (pg. 21, ll. 21-24; col. 14, ll. 61-63). In one embodiment in **Jayasena** *et al.* the aptamer does not hybridize with the ligand beacon if the target is not bound to it. The target (Applicant's ligand) induces a conformational change in the aptamer upon binding thereto which conformational change induces the ligand beacon to act as described. Thus, the reporter in **Jayasena** *et al.* is not associated with the aptamer in any way prior to the target binding to it and is bound to the aptamer via hybridization and not covalent binding.

In the preferred embodiment in **Jayasena** *et al.* the aptamer will hybridize to the ligand beacon if the target is not present (pg. 26, ll. 4-19; col 17, ll. 60 to col. 18, ll.

16). Again this causes the ligand beacon to act as described. This aptamer has a greater binding affinity for the target, so in the presence of target, the aptamer preferentially binds to the target. The amount of aptamer available to hybridize to the binding ligand and transduce the hybridization to fluorescence inversely correlates to amount of target available to bind the aptamer. Thus, the free aptamer and not the target bound aptamer induces the conformational change.

Neither of these embodiments anticipates Applicant's amended claims 1 and 15. Dependent claims 11 and 23 are canceled. Claims 6-10 and 12 depend from amended claim 1 and claims 19-22, 24-25 and 28 depend from amended claim 15. **Jayasena** *et al.* ('31276) and **Jayasena** *et al.* ('286) do not anticipate these claims for the same reasons in overcoming the rejection of **Pitner** *et al.* presented *supra*.

Absent teachings of a non-nucleic acid ligand transducing the conformational change of a nucleic acid specific for the ligand to an increase in fluorescence of a reporter/dye contained therein, **Jayasena** *et al.* ('31276) and **Jayasena** *et al.* ('286) do not anticipate the amended claims. Accordingly, in view of the amendments and arguments presented herein, Applicants respectfully request that the rejection of claims 1, 6-12, 15, 19, 23, 25, and 28 under 35 U.S.C. §102(b) be withdrawn.

The 35 U.S.C. §103(b) rejections

Claims 20-22, and 24 are rejected under 35 U.S.C. §103(a) as being unpatentable over **Jayasena** *et al.* (U.S. 6,531,286) in view of **Szostak** *et al.* (U.S. 5,631,146). Applicant respectfully traverses this rejection.

Jayasena *et al.* ('286) is discussed *supra*. **Szostak** *et al.* teach single-stranded DNA molecules which bind adenosine or an adenosine-5'-phosphate and methods for producing and isolating them. Claims 20-22 and 24 depend from amended independent claim 15. Claims 20-22 and 24 limit the aptamers and ligands used in the instant method.

Applicants maintain that **Jayasena** *et al.* ('286) do not teach all the claim elements of the instant invention. Combining **Szostak** *et al.* with **Jayasena** *et al.* ('286) does not remedy that defect. One of ordinary skill in the art may be motivated to limit the nucleic acid ligand or aptamer, as taught in **Jayasena** *et al.* ('286) to an anti-adenosine RNA aptamer or an anti-adenosine DNA aptamer and the target to adenosine, as taught by **Szostak** *et al*, however, this combination does not render the instant invention obvious.

The combination of '286 and **Szostak** *et al.* does not suggest to nor motivate one of ordinary skill in the art to modifiy the ligand beacon assay so that a non-nucleic acid target, absent the nucleic acid ligand, could bind to and induce the conformational change of the ligand beacon with resulting signaling of a fluorescent moiety within the ligand beacon. Nor is there a reasonable expectation of success in such modification because '286 teaches that ligand beacons are nucleic acid molecules, labeled with an energy transfer pair, that can specifically hybridize to a nucleic acid ligand which causes the conformational change in the ligand beacon (pg. 21, ll. 6-7; col. 14, ll. 37-43). Such modification would render the prior art unsatisfactory for its intended purpose.

Jayasena *et al.* ('286) require an aptamer that is specific for a target and that hybridizes to the ligand beacon. The ligand beacon will only change conformation if the aptamer, with or without the target, hybridizes therewith. The specific teaching of a nucleic acid ligand hybridizing with the nucleic acid containing the energy transfer pair

teaches away from Applicant's claimed element that the ligand is a molecule binding to a nucleic acid aptamer containing a reporter/dye and that the ligand is not a nucleic acid sequence. Therefore, the invention as a whole was not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicant respectfully requests that the rejection of claims 20-22 and 24 under 35 U.S.C. §103(a) be withdrawn.

This is intended to be a complete response to the Office Action mailed March 30, 2004. Applicants believe the pending claims are in condition for allowance. If any issues remain, the Examiner is respectfully requested to telephone the attorney of record signing the instant document for immediate resolution. Applicants include a Petition for Extension under 37 C.F.R. 1.136. Please debit the $55 extension fee under 37 C.F.R. 1.17(a) or any applicable fees from Deposit Account No. 07-1185 upon which the undersigned is allowed to draw.

Respectfully submitted,

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